Environmental Risk Research on Pest Resistant LMO

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1. Purpose of Research

- The grain import of Korea sharply increases owing to the recent decline of domestic food self sufficiency rate, At the same time, as the relatively low cost LMO import increases, the people's concern over safety of LMO equally goes up.
- While the insecticidal proteins such as Cry or Vip3Aa are used in the typical pest resistant LMO, the present research is intended to establish a method isolating protein in massive scale from colon bacillusF in current year of research implementation, and closely examine the fundamental molecular & biological characteristics of pure isolated Vip3Aa protein.
- Also, the simple risk experiment on indigenous soil microorganisms & molds is to be conducted using the pure isolated Vip3Aa protein as a preliminary research assessing the risk.

2. Major Aspects

1) Gene cloning for isolation of Vip3Aa genes and protein overexpression from bacillus.

- Insertion of Vip3Aa genes into overexpressed vector is identified through the sequence analysis after isolating the B. thuringiensis Vip3Aa genes using Polymerase chain reaction (PCR) process and inserting them into pET28a, the colon bacillus and protein expression vectors.

2) Overexpression of Vip3Aa recombinant protein in colon bacillus is verified.

- Processed the IPTG, an overexpressed protein substance, with diverse concentrations after cultivating colon bacillus introduced with overexpressed Vip3Aa genes up to the 30℃ OD 0.6 condition. In order to check the expression level of Vip3Aa protein, isolate the entire proteins from colon bacillus and conducted the electrophoresis.
- As a result of investigation on the volume of Vip3Aa protein expression, the largest expression of protein with IPTG 0.3mM was identified, as well confirming that the water soluble Vip3Aa protein can be isolated.

3) Establishment of Optimized Process for Massive Isolation of Vip3Aa Protein

- Process of isolating the Vip3Aa protein in mass from colon bacillus massive cultivated is established, as the overexpression of Vip3Aa protein is verified in the water dissolving section of colon bacillus.
- Kept the massive cultivated colon bacillus in storage under -80℃ temperature condition after melting in phosphate buffered saline (PBS) buffer, melting the frozen colon bacillus and then crushed the cells with ultrasonicator. Isolated the crushed colon bacillus with ultracentrifuge, filtered the solution, isolate the Vip3Aa protein using affinity chromatography method, and then verified the size and purity through SDS-PAGE.

4) Mass Spectrometry Movement of Pure Isolated Vip3Aa Protein

- Verified the fact that the protein pure isolated from colon bacillus is Vip3Aa protein through mass spectrometry (MALDI-TOF) analysis. As a result of PMF analysis conducted by cutting the 88kDa of protein band off expected after executing the SDS-PAGE experiment on pure isolated protein, the moving protein pieces examined coincided with the amino acid sequence of Vip3Aa protein.

1) Morphological Characteristic Analysis of Vip3Aa Protein

- Observed the molecular morphology of Vip3Aa using the Native-PAGE & FPLC analysis in order to identify the morphological characteristics of Vip3Aa recombinant protein. As a result, interestingly enough, it was identified that the Vip3Aa constituted very big size of homo-digomer molecules, not the monomer molecules of low molecular shape.

5) Insecticidal Test on Vip3Aa Protein with Domestic Target & Non-target Insects

- Insecticidal tests on target & non-target larvae were conducted in order to identify whether the Vip3Aa protein is under activated state or not.
- Sprinkled 1mg/ml of Vip3Aa proteins to host plants of insects preparing the Vip3Aa protein and buffer (control group), and dried them up. Observed for 5 days upon putting 5 each of Eligma narcissus & Hyphantria cunea lavae in respectively.
- As a result, the death of about 60% of Eligma narcissus lavae treated with Vip3Aa proteins was observed but no difference was shown with the Hyphantria cunea lavae.
- Also, no pesticidal effect appearance on Tenebrio molitor but showing the pesticidal effect on Plodia interpunctella were observed with Vip3Aa.

6) Environmental Risk Assessment of Vip3Aa Protein against the Korea Domestic Bacteria & Molds

- As a result of treating highly concentrated Vip3Aa proteins under a specific condition (variation of pH) with diverse bacterial groups, the impediment on Bacillus magaterium & Proteus vulgaris growth was identified but not able to confirm the risk of Vip3Aa proteins with molds.
- Appearance of risk under specific condition and biota with Vip3Aa proteins was verified through above mentioned result.

3. Results

- The Vip3Aa pesticidal protein overexpressing condition is established using colon bacillus, setting the optimized massive protein isolation technology up by effectively isolating the great quantity of Vip3Aa Recombinant proteins.
- The result of preliminary pesticidal test conducted on Korean indigenous target & non-target insects using Vip3Aa proteins obtained employing the optimized pure isolation technique showed the risk with specific insect group.
- The result of preliminary environmental risk research conducted with microorganisms recognized as Korea indigenous non-target microorganisms of Insect resistant Vip3Aa proteins showed risk with the specific bacteria.

4. Applications

- The massive isolation technology developed and established with Vip3Aa pesticidal protein on colon bacillus is applicable to the massive amount of other pesticidal protein isolation.
- The information including designation of insect category, concentration of Vip3Aa proteins, protein exposure time & etc. are provided for environmental risk assessment, which can be utilized in setting the direction and guidelines up in future researches to be conducte for accurate risk assessment.
- Risk assessment standard can be prepared when examining the for LMO imported into Korea for approval, and the subjective risk data for approved LMO can be provided through the result obtainable from concrete Korea domestic natural ecosystem impact researches tp be conducted in the future..
- For the Korea domestic environmental risk test of pesticidal proteins, the accurate risk assessments depending on the differences of diverse environment changes & territory, soil, temperature, moisture, pH & other conditions are required.

5. Source

- National Institute of Ecology (www.nie.re.kr)